Utilizing Composted Substrate Upgrades Yield and Quality of Oyster Mushroom Grown on Rice Straw

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ABSTRACT:

A study was conducted in the mushroom research and production laboratory, Department of Horticulture, Faculty of Agriculture, Assiut University during Oct. to April 2011 and 2012. Production of oyster mushroom (*Pleurotus columbinus*) fruiting bodies was assessed for different recipes of mixed composted materials and raw rice straw substrate (w/w). These recipes were: 1) raw rice straw (RRS) mixed with 5% composted materials (CM), 2) RRS mixed with 10% CM, 3) RRS mixed with 15% CM, 4) RRS mixed with 25% CM, 5) RRS mixed with 50% CM and 6) 100% RRS. The studied parameters were fruiting bodies' yield, spent weight, biological efficiency, days lapsed to visible pinheads formation and fruiting body cap and stem diameter, thickness and weight. Data showed a magnificent impact of substrate preparation on oyster mushroom productivity. There was a significant progressive upgrading in all studied parameters of mushroom growth and crop outcome with increasing the percentage of CM mixed with the RRS substrate up to 15%. Utilizing CM at 25% significantly downgraded these parameters. No mushroom growth was observed at all when cultivated in medium contained 50% CM. Instead, molds of different colors grown on that latter substrate mixture. The recipe containing 15% CM distinctly gave the uppermost fruiting bodies' yield and quality. This recipe exhibited increase in fruiting bodies crop outcome ranged from 120% to 170% relative to growing on sole RRS. In spite of oyster mushroom capability to biodegrade lignocellulosic materials, this substrate may not provide all nutrients required, especially nitrogen, for its optimum growth. In this regard, this study suggests that composted materials hold a great promise for development of oyster mushroom industry.

Keywords: fruiting bodies, edible fungi, lignocellulosic materials, macrofungi, *Pleurotus spp*., primary decomposer.

INTRODUCTION

The cultivated oyster mushroom (*Pleurotus spp*.) includes a number of different species; *Pleurotus ostreatus, Pleurotus sajor-caju, P. columbinus, Pleurotus cystidus, Pleurotus citrino-pileatus* and *Pleurotus flabella-tus*. However, *P. ostreatus* (a wood-destroying fungus) is the most frequently cultivated species of the genus *Pleurotus* **(Kong, 2004).** It is widespread in the temperate zones representing one of the largest groups of the cultivated edible mushrooms in the world (**Mendez et al., 2005; Sarangi et al., 2006; Sher et al., 2010**). China is the major producer of oyster mushroom. The production of this mushroom species is estimated to be 25% of total world production of cultivated mushrooms (**http://www.isms.biz/edibles.htm 2/6/2009**). The oyster mushrooms are nutritionally and gastronomically important (**Sarangi et al., 2006**).

Appropriate preparation of the substrate is crucial for the production of maximized yield of oyster mushrooms (**Choi et al., 2009; Obodal and Johnson, 2002; Soliman, 2011**). Oyster mushrooms (*Pleurotus* spp.), unlike button mushroom (*A. bisporus*), are primary decomposers. They can break down and absorb the components of substrate materials that have not been composted or degraded. Therefore, cultivation of oyster mushroom is considered to be a simple, low cost and environmentally friendly technology for the utilization of rural and agro- industrial residues in the developing countries **(Kirbag and Akyüz, 2008).** However, Korean growers have found that fermented substrate materials produce high yield and quality oyster mushrooms (**Choi, 2004**). **Obodal and Johnson (2002)** showed that composted *T. Scleroxylon* sawdust mixed with other substrates significantly increased the yield of *P. ostreatus*.

Substrate materials in nature have microorganisms attached to their surfaces. The initial microorganisms that exist come mainly from the soil. These microorganisms are suppressed on dry material. when moistened, microorganisms can propagate and the nutritive substances of the substrate are accumulated in the form of protein. Thus fermentation in mushroom cultivation can be defined as the conversion of the nutrients of substrates by microorganisms into proteins (**Choi, 2004**). By pasteurization of substrate, microorganisms are killed and protein can be utilized by growing mushroom. An assessment of oyster mushroom (*Pleurotus columbinus*) productivity when cultivated on composted and raw rice straw substrate recipe utilized at different mixed portions is presented in this study.

MATERIALS AND METHODS

The current study was conducted in the mushroom research and production laboratory, Department of Horticulture, Faculty of Agriculture, Assiut University

during Oct. to April 2011 and 2012. Production of oyster mushroom (*Pleurotus columbinus*) fruiting bodies was assessed for different recipes of mixed composted materials and raw rice straw substrate (w/w). In a preliminary trial, four treatments were designated. These were: 1) 100% raw rice substrate (RRS) (control), 2) 50 % RRS mixed with 50% composted materials (CM) (w/w), 3) 75 % RRS mixed with 25% CM, and 4) 100% CM. Based on this preliminary assessment, the treatments of the substrate recipe were modified to be: 1) RRS mixed with 5% CM, 2) RRS mixed with 10% CM, 3) RRS mixed with 15% CM, 4) RRS mixed with 25% CM, 5) RRS mixed with 50% CM (negative control) and 6) 100% RRS (positive control). Spawn of *Pleurotus columbinus* mushroom used in this study was obtained from Agricultural Research Center, Food Technology Research Institute, Giza.

Preparation of composted substrate materials

Moistened chopped raw rice straw was mixed with chicken manure and soil (4:1:1, v/v). The soil was added to enrich the materials with biodegrading mesophile microorganisms(**Choi, 2004**). Raw rice straw was used as a carbon source for biodegrading mesophile microorganisms. The mixture was piled up outdoors and moistened. The heap was kept for 2 weeks. Then the pile was turned weekly to provide fresh air and prevent overheating. The composted material was continued under this process for 6 weeks. The mature compost was then was autoclaved under 1.2 kg/cm^2 at 121ºC for 20 min before use in preparation of the different substrate recipes.

Preparation of substrate recipes and spawn inculcation

The raw rice substrate was moistened thoroughly by soaking overnight in water. The substrate was pasteurized for 2 h in hot water at 80°C **(Bahukhandi and Munjal, 1989; Balasubramanya and Kathe, 1996)**. The pasteurized substrate was left to cool down and to drain excess water. Subsequently, the substrate was thoroughly mixed with the composted materials and checked to assure average mean moisture of about 70%. The prepared substrate was manually packaged into 20 X 40 cm clear polyethylene bags of mean thickness 0.2 mm containing 1 kg moistened substrate. The spawn was inoculated at rate of 5% (based on wet mass of the substrate).

Culture conditions for spawn running and fruiting bodies formation

The inoculated substrate was incubated for spawn running at 24-28^o C in the darkness for 3 weeks. The mushroom cultures were subsequently transferred into fruiting room for basidiocarp formation.Polyethylene bags were removed and the cultures were kept at 22±1^o C under light provided by cool white fluorescent tubes for 12 h a day. Electric fans were used 4 h a day during

incubation for basidiocarp formation to provide homogenous ventilation condition in the incubation room. The bags' moisture was maintained by spraying with water 2 times a day during the whole cropping period. Mushroom fruiting bodies were harvested about a week after pinheads formation that was as soon as the gills were well formed and while the edge of the mushroom is still curled under. All experiments were conducted in randomized complete-blocks with 4 replicates. Each treatment was presented by 5 culture bags within replicate.

The data were recorded for days lapsed to visible pinhead (primordia) formation, fruiting bodies yield (g/100 moistened substrate), spent weight (g), biological efficiency (%), weight (g), diameter (cm) and thickness (mm) of both fruiting body and stem. Biological efficiency (BE) was calculated as follows: BE (%) = (weight of fresh mushroom fruiting bodies/ weight of dry substrate) x 100 (**Ahmed, 1995; Kirbag and Akyüz. 2008**).All data were subjected to analysis of variance(**Gomez and Gomez, 1984**) and means were compared using "The Least Significant Difference" (LSD) Test at 0.05 probability level.

RESULTS

The substrate recipes containing 50%, 75% and 100% composted material (CM) showed no mushroom ramification and subsequently no fruit body formation in the preliminary assessment. Meanwhile, mushroom fungus ramification and fructification were observed on the sole raw rice straw (RRS) substrate. Fruit bodies yield of oyster mushroom (*Pleurotus columbinus*) grown on RRS mixed with 5% composted material (CM), in contrast to sole raw rice straw (RRS) substrate, exhibited significant increase in the first trial but not in the second one (Table 1 A). The substrate recipe containing 10% CM significantly elevated fruit bodies yield in both trials. When grown on substrate recipe containing 25% CM, mushroom showed tendency to produce lower yield in the first trial and clearly significant reduction in fruit bodies yield in the second trial. Consistently, mushroom grown on substrate recipe containing 15% CM produced fruit bodies' yield significantly surpassing all the other studied treatments.

Data obtained for spent weight substantiated those of the fruit bodies' yield (Table 1 B). Spent weight was the least for mushroom grown on the substrate recipe composed of 85% RRS and 15% CM. This indicates that the fungus degraded larger amount of components of this substrate recipe. Mushroom grown on recipe containing 5% CM utilized greater amount of the substrate than the one grown on RRS (control). Meanwhile, Greater amount of the substrate recipe containing 10% CM was degraded by the fungus than that one containing 5%CM. However, the utilized quantity of the substrate recipe

containing 25% did differ from that of the RRS (positive control). Due to the highest biodegradation for the substrate recipe containing 15% CM, it exhibited the highest biological efficiency (Table 1 C). The other substrate treatments, except 25% and 50% CM, showed inconsistence with regard to the significance of the difference in biological efficiency from the RRS substrate. Apparently, The substrate recipe containing 25% or 50% CM was inferior to the RRS substrate regarding the biological efficiency.

As a result of the superior capability for biodegradation of substrate having 15% CM, the fungus formed its pinheads exceptionally earlier (Table 1 D). Differently, the days lapsed to visible pinhead formation was similar for the mushroom grown on 0%, 5%, 10% and 25%. Obviously, the availability of nutrient components from the substrate for the mushroom grown on the recipe containing 15% CM enabled developing fruit bodies with caps and stems of largest diameter, heaviest weight and more thickness (Tables 2 and 3; A, B and C) (Fig. 1).

DISCUSSION

The present study shows a magnificent impact of substrate preparation on oyster mushroom productivity. Noticeable, there was a significant progressive upgrading in all studied parameters of mushroom growth and crop outcome with increasing the percentage of composted materials mixed with the raw substrate up to 15%. Utilizing the composted materials at 25% apparently downgraded these parameters. No mushroom growth was observed at all when cultivated in medium contained 50% or more of composted materials. Instead, molds of different colors grown on that latter substrate mixture.

The main components of the lignocellulosic substrates are cellulose, hemicellulose and lignin. Oyster mushrooms can biodegrade lignocellulosic materials to get its carbon requirements. However, mushrooms need also nitrogen and inorganic compounds. The substrate material utilized alone for cultivation of oyster mushroom sometimes cannot provide enough nitrogen required for optimal growth (**Soliman, 2011; Soliman, et al., 2011**). Therefore, supplements such as rice and wheat bran are added as a nitrogen source (**Soliman, 2011**). Composting raw substrate materials converts them into a superior nutritional source especially protein for mushroom (**Rajarathnam & Bano, 1989**) through the actions of a succession of microorganisms. Unlike the end up result of our study, composted materials from raw substrate without supplements can be utilized wholly as substrate for cultivation of oyster mushroom and Korean growers have found it definitely improve yield and quality of oyster mushroom crop outcome **(Choi, 2004).** In their method, raw substrate is moistened and left for composting by the microorgan-

isms naturally subsist attached to the surface of the dry substrate.

Here we prepared the composted materials from rice substrate mixed with chicken droppings. Diminutive information has been reported on use of chicken manure supplement to substrates in production of mushroom. As reported by **Baysal et al. (2003**) increased supplement of chicken manure to waste paper substrate had a negative effect on growing mushroom. The chicken manure is rich in nitrogen, in addition to, phosphorus, potassium and calcium. Also, rice straw (**Choi, 2004**) normally contains more total N than other common substrates such as cotton wastes, wheat straw and sawdust. Through the composting process, nitrogen is converted into ammonia nitrogen and if available nitrogen increases, ammonia nitrogen also increases. Oyster mushroom yield decreases when the ammonia concentration is higher than 68 ppm as well as when total nitrogen is smaller than the optimal amount (**Choi, 2004**). The used compost here may have high total N and mixing it with raw substrate could have adjusted the total N to the suitable level for mushroom growth. This may provide an explanation for the results obtained in our current study in contrast to the Korean method.

Contamination of the pasteurized substrate may occur if high temperature (< 60ºC) and time (< 1-2h) used during pasteurization. Probably this is due to the partial breakdown of cellulose and hemi-cellulose, making them available to competitor microorganisms. Pasteurization at 90°C could make cellulose more available (**Sturion and Oetterer, 1995),** due to the partial destruction of the lignin-cellulose bonds, favoring substrate contamination. The microorganism species that may compete with *Pleurotus spp.* after pasteurization with hot water (80°C for 2 hours) includes the fungi *Penicillium spp*. and *Trichoderma spp*. (green mold) (**Balasubramanya and Kathe, 1996**). Additionally, it is worth to mention that lack of the competition due to the restrained growth of mushroom can offer a chance for molds to grow. We conducted pasteurization at 80°C and this may be accounted for the contamination observed especially when mushroom growth is ceased.

Mixing composted material with raw rice substrate at rate of 15% elevated the biological efficiency of growing mushroom. The crop outcome exhibited an increase ranged from 120% to 170% relative to growing on sole raw substrate. This improved fruit bodies' yield was accompanied with magnificent enhanced weight and size for the harvested fruit bodies. This substrate recipe produced mushroom crop earlier by 5 days, on average, than the cultivation on raw rice straw. It is well documented that rice straw is the most productive among other common substrates for the production of oyster mushroom

(**Zhang et al., 2002).** The composted material utilized in this study may save wheat bran (tradition supplement to substrates for production of oyster mushroom) for bakery process. Composting described here is simple and relatively inexpensive when considering the mushroom crop increment. **In conclusion**, composting holds a promise in developing oyster mushroom industry. Under the condition of this study, a recipe of mixed composted material (chicken manure and rice straw) with raw rice straw at rate of 15% is proposed for elevating yield and quality of oyster mushroom.

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Table (1): Means of fruiting bodies' yield, spent weight, biological efficiency and days lapsed to visible pinheads formation for oyster mushroom (*Pleurotus columbinus*) cultivated on raw rice straw (RRS) mixed with 15% composted materials (CM) [1]

Treatments [2]	1st Trial	2nd Trial
(A)- Fruiting bodies yield (g/kg substrate)		
0	148.357	170.724
5	175.661	171.136
10	206.007	186.249
15	248.169	201.923
25	133.251	136.036
50	0.0[3]	0.0
$LSD_{0.05}$	19.732	18.065
(B)- Spent weight (g)		
0	668.500	691.750
5	466.667	504.667
10	401.083	400.417
15	267.083	246.167
25	685.500	689.917
50	776.5	817.6
$LSD_{0.05}$	33.7	49.6
(C)- Biological Efficiency (%)		
0	59.3	68.3
5	70.3	68.5
10	82.4	74.5
15	99.3	80.8
25	53.3	54.4
50	0.0	0.0
$LSD_{0.05}$	7.9	7.2
(D)- Days lapsed to visible pinheads (primordia) formation		
0	34.5	34.5
5	34.3	34.3
10	34.2	35.2
15	29.6	29.8
25	34.6	35.4
50	0.0	0.0
$LSD_{0.05}$	0.4	1.2

[1] composted materials (CM) were prepared utilizing raw rice straw (RRS) mixed with chicken manure and soil (4:1:1, v/v).

[2] percent composted materials (CM) added to raw rice straw (RRS).[3] no mushroom growth occurred.

Table (2): Means of fruiting body cap diameter, thickness and weight for oyster mushroom (*Pleurotus columbinus*) cultivated on raw rice straw (RRS) mixed with 15% composted materials (CM) [1]

Treatments[2] [2]	1st Trial	2nd Trial
Average diameter of fruiting body cap (mm)		
0	86.8	91.5
5	102.8	99.3
10	115.3	116.5
15	136.8	130.8
25	92.5	85.0
50	0.0 [3]	0.0
$LSD_{0.05}$	5.1	8.9
Average thickness of fruiting body cap (mm)		
0	7.3	8.3
5	8.8	9.0
10	8.9	9.8
15	14.7	13.7
25	7.6	8.1
50	0.0	0.0
$LSD_{0.05}$	2.3	1.7
Average weight of fruiting body cap (g)		
0	7.318	7.603
5	7.728	8.504
10	8.761	9.061
15	9.853	9.625
25	7.172	7.352
50	0.0	0.0
$LSD_{0.05}$	1.340	1.455

[1] composted materials (CM) were prepared utilizing raw rice straw (RRS) mixed with chicken manure and soil (4:1:1, v/v).
[2] percent composted materials (CM) added to raw rice straw (RRS).
[3] no mushroom growth occurred.

Table (3): Means of fruiting body stem weight, length and diameter for oyster mushroom (*Pleurotus columbinus*) cultivated on raw rice straw (RRS) mixed with 15% composted materials (CM) [1]

Treatments [2]	1st Trial	2nd Trial
	Average weight of stem (g)	
0	2.905	3.025
5	3.255	3.227
10	3.387	3.622
15	3.975	3.932
25	2.988	3.037
50	0.0 [3]	0.0
$LSD_{0.05}$	0.141	0.132
	Average length of stem (cm)	
0	2.5	2.5
5	2.8	2.8
10	3.1	3.3
15	4.1	4.2
25	2.5	2.5
50	0.0	0.0
$LSD_{0.05}$	0.2	0.2
	Average stem diameter (mm)	
0	7.3	9.9
5	8.6	12.1
10	9.9	14.2
15	24.0	25.7
25	9.9	10.5
50	0.0	0.0
$LSD_{0.05}$	4.7	2.4

[1] composted materials (CM) were prepared utilizing raw rice straw (RRS) mixed with chicken manure and soil (4:1:1, v/v).
[2] percent composted materials (CM) added to raw rice straw (RRS).
[3] no mushroom growth occurred.

Fig. 1. Photographs show mushroom (*Pleurotus columbinus*) production utilizing substrate recipe composed of 85% raw rice straw (RRS) and 15% composted materials (rice straw and chicken manure): 1) Cluster of oyster mushroom fruiting bodies and 2) size (diameter) of a representative fruiting body.

ملخص الدراسه باللغه العربيه

استخدام الكمبوست يحسن محصول وجودة عيش الغراب المحارى المزروع على قش الارز

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اجريت هذة الدراسة فى معمل بحوث وانتاج عيش الغراب المحارى- قسم البساتين كلية الزراعة - جامعة اسيوط ، خلال الفترة من اكتوبر الى ابريل (٢٠١١-٢٠١٢) واستخدم فيها عيش الغراب نوع كلومبينس والذى تم زراعتة على بيئات مختلفة من مخلوط الكمبوست وقش الارز وكانت المعاملات هى: ١- قش الارز مضاف له ٥% كمبوست ، ٢- قش الارز مضاف له ١٠% كمبوست ، ٣- قش الارز مضاف له ١٥% كمبوست ، ٤- قش الارز مضاف له ٢٥% كمبوست ، ٥- قش الارز مضاف الية ٥٠% كمبوست ، واستخدم قش الارز الخام (الغير معامل) بدون اضافة الكمبوست كمعاملة للمقارنة. وقد تم اعداد الكمبوست قبل الزراعة من مخلوط من زرق الدواجن والتربة الطينية وقش الارز وكان الهدف من الدراسة هو التعرف على انسب مقادير خلط الكمبوست مع قش الارز غير المعامل وتم تسجيل بيانات عن : المحصول الكلى ، وزن الاكياس بعد جمع المحصول،الكفاءة الحيوية، عدد الايام لظهور الرؤوس، متوسط قطر وسمك ووزن القبعة وكذلك متوسط وزن وطول وقطر الساق. وقد اظهرت النتائج زيادة تدريجية فى كمية المحصول وجودة الثمار مع زيادة نسبة الكمبوست فى بيئة الزراعة وذلك حتى ١٥% مقارنة بالكنترول (المعاملة التى لم يتم لها الاضافة) الا انة وجد تناقص عند زيادة نسبة الكمبوست المضافة عن ١٥% ولم ينمو عيش الغراب المحارى مطلقا عند اضافة الكمبوست بنسبة ٥٠% ، وفى تلك الحالة لوحظ نمو بعض الاعفان المختلفة واكثرها ذات الوان خضراء أو صفراء وقد بلغت الزيادة فى المحصول عند استخدام ١٥% مادة عضوية ١٢٠% الى ١٧٠% مقارنة بالكنترول .